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3 KINTERSTANDANCE STANDANCE STANDANC

T or A

23

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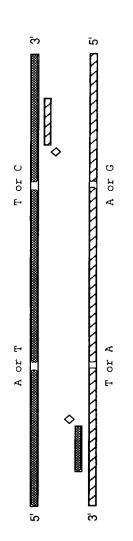
0

2

A or G

PCR/ LDR

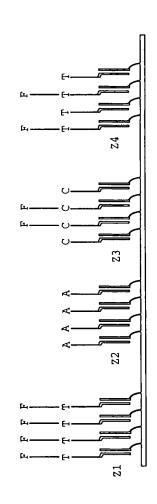
 PCR amplify region(s) containing mutations using primers, dNTPs and Taq polymerase.



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2. Perform LDR using allele-specific LDR primers and thermostable ligase. O Allele specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.

 Capture fluorescent products on addressable array and quantify each allele.

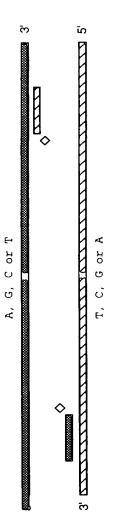


Homozygous: T allele only.

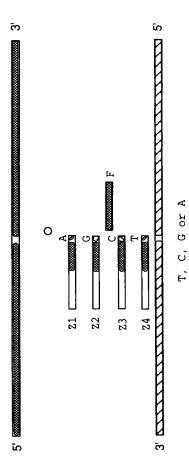
Heterozygous: C and T alleles.

PCR/LDR

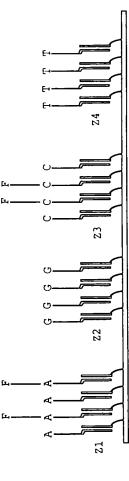
 PCR amplify region(s) containing mutations using primers, dNTPs and Taq polymerase.



2. Perform LDR using allele-specific LDR primers and thermostable ligase. O Allele specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.



 Capture fluorescent products on addressable array and quantify each allele.

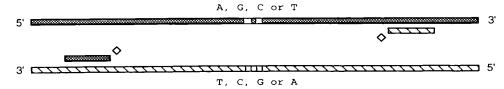


Heterozygous: A and C alleles.

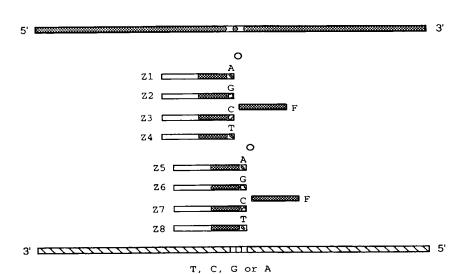
IFIIG. 4

PCR/LDR: N arby alleles

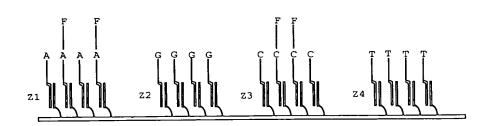
 PCR amplify region(s) containing mutations using primers, dNTPs and Taq polymerase.◊



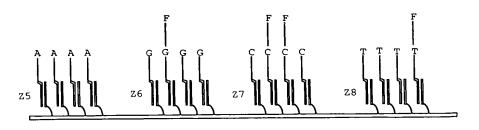
 Perform LDR using allele-specific LDR primers and thermostable ligase. O Allele specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.



 Capture fluorescent products on addressable array and quantify each allele.



Heterozygous: A and C alleles.



Heterozygous: G,C, and T alleles.

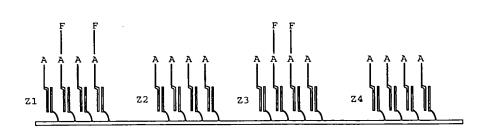
PCR/ LDR: Insertions and Deletions

- PCR amplify region(s) containing mutations using primers, dNTPs and Taq polymerase.
- A5-8 Variable Deletion in (CA)n

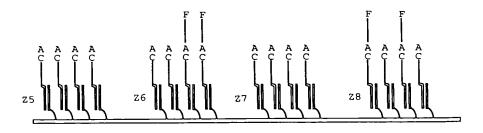
 5'

 T5-8 Variable Deletion in (GT)n
- Perform LDR using allele-specific LDR primers and thermostable ligase. O Allele specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.

 Capture fluorescent products on addressable array and quantify each allele.



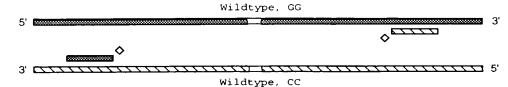
Heterozygous: A5 and A7 alleles.



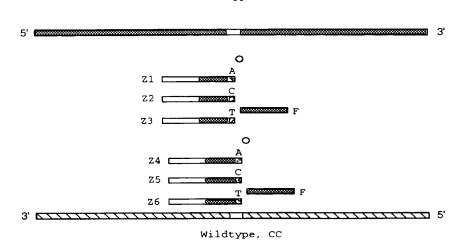
Heterozygous: (CA)5 and (CA)3 alleles.

PCR/ LDR: Adjacent alleles, cancer detection

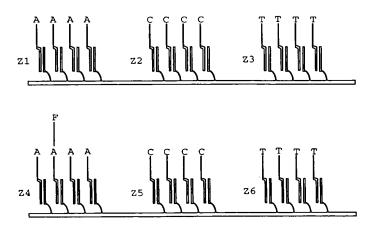
 PCR amplify region(s) containing mutations using primers, dNTPs and Taq polymerase.



 Perform LDR using allele-specific LDR primers and thermostable ligase. O Allele specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.



 Capture fluorescent products on addressable array and quantify each allele.



Gly to Asp mutation

PCR/ LDR: Nearby alleles

- PCR amplify region(s) containing mutations using primers, dNTPs and Taq polymerase.
- A, G, C or T

 A, G, C or T

 5'

 T, C, G or A

 T, C, G or A

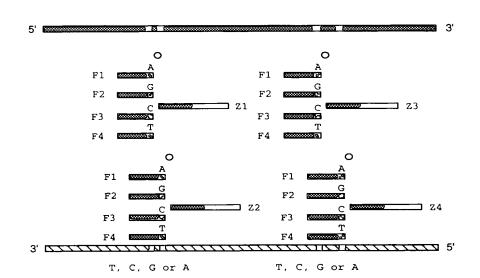
 A, G, C or T

 A, G, C or T

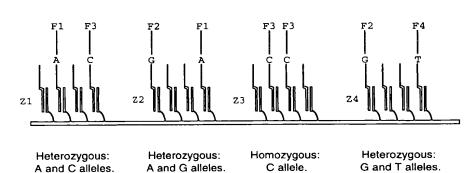
 T, C, G or A

 A, G, C or T

 T, C, G or A
- Perform LDR using allele-specific LDR primers and thermostable ligase. O Allele specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.



 Capture fluorescent products on addressable array and quantify each allele.



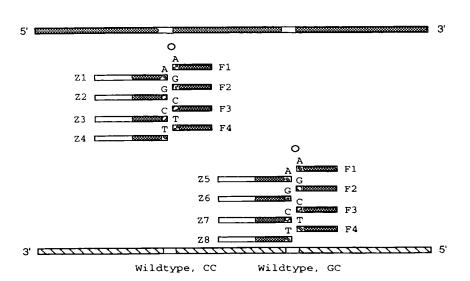
PCR/ LDR: Adjacent and Nearby alleles

- PCR amplify region(s) containing mutations using primers, dNTPs and Taq polymerase. ◊
- Wildtype, CG

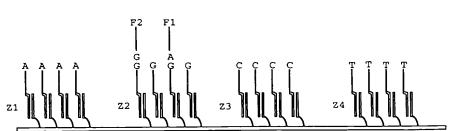
 Wildtype, CG

 Wildtype, CC

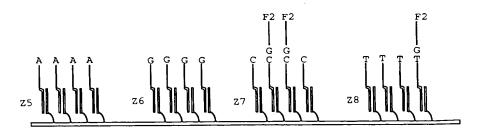
 Wildtype, CC
- Perform LDR using allele-specific LDR primers and thermostable ligase. O Allele specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.



 Capture fluorescent products on addressable array and quantify each allele.



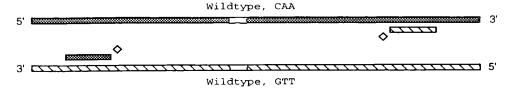
Heterozygous: Gly and Glu alieles.



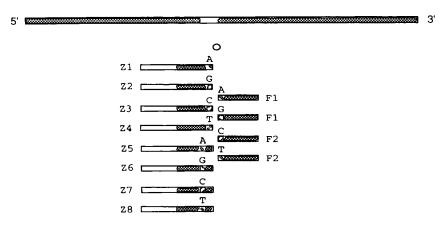
Heterozygous: Arg and Trp alleles.

PCR/ LDR: All alleles of a single codon

 PCR amplify region(s) containing mutations using primers, dNTPs and Taq polymerase.

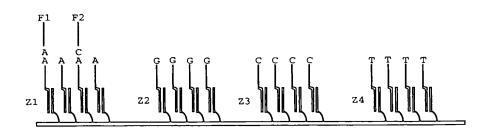


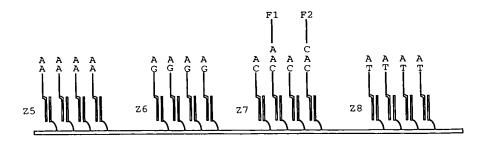
 Perform LDR using allele-specific LDR primers and thermostable ligase. O Allele specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.



3' Wildtype, GTT

 Capture fluorescent products on addressable array and quantify each allele.





Heterozygous: Gln and His alleles.

$$X = -OH$$
 X^* Y^{-PEG}
 X^* Y^{-PEG}
 $X,Y = -OH$ Y^* Y^{-PEG}
 X^* $Y^* = -O(C=O)Z$
 Y^{-PEG}
 Y^{-PEG}

W = protecting group, e.g. Boc, Fmoc

Z = activating group, e.g. imidazole (Im), p-nitrophenol (OPnp),hydroxysuccinimide (OSu), pentafluorophenol (OPfp)

PEG = oligo or poly(ethylene glycol), backbone $(CH_2CH_2O)_n$ $\underline{n} = 6$ to 200 (can also be grown by anionic polymerization with)

WSC = water soluble carbodiimide

Functional group transformations/activation (as needed), $X \rightarrow X^*$, $Y \rightarrow Y^*$

$$\begin{array}{ccc} -\text{OH} & \longrightarrow -\text{O(CH}_2)_n\text{CO}_2\text{H} & \text{n} = 1, 2 \\ -\text{OH} & \longrightarrow -\text{O(C=O)NHCH}_2\text{CO}_2\text{H} \\ -\text{OH} & \longrightarrow -\text{O(C=O)CH}_2\text{NH}_2 \end{array}$$

 $-OH \longrightarrow -O(C=O)Im$

 $-OH \longrightarrow -O(C=S)SCH_2(C=O)NH_2$

 $-CO_2H \longrightarrow -(C=O)NH(CH_2)_nNH_2$ $-CO_2H \longrightarrow -(C=O)Z$

 $-NH_2 \rightarrow -NH(C=O)(CH_2)_nCO_2H$ n = 2, 3

Covalent linkage, X* + Y*

$$-CO_{2}H + H_{2}N - + WSC + HOSu \longrightarrow -(C=O)NH - OH + Im(C=O)Im + H_{2}N - O(C=O)NH - OH + Im(C=O)Im + H_{2}N - O(C=O)NH - OH + N \longrightarrow CI \longrightarrow N \longrightarrow CI + H_{2}N - OH - N \longrightarrow N \longrightarrow N - NH - OH + O=C=N - O(C=O)NH - O(C=S)SCH (C=O)NH + H_{2}N \longrightarrow O(C=S)NH - O($$

$$-O(C=S)SCH_2(C=O)NH_2 + H_2N_- \longrightarrow -O(C=S)NH_-$$

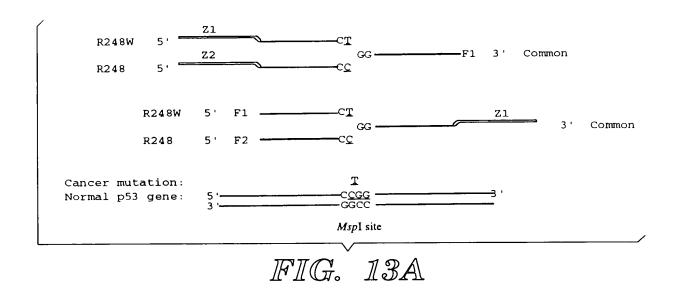
$$-OH + O=C=N- \longrightarrow -O(C=O)NH-$$

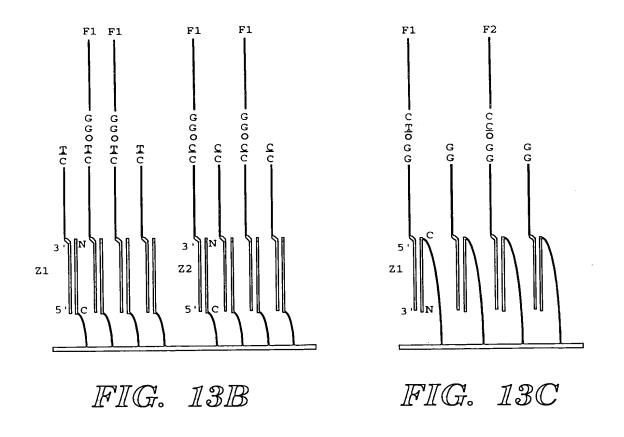
$$-O(C=S)SCH_2(C=O)NH_2 + H_2N- \longrightarrow -O(C=S)NH-$$

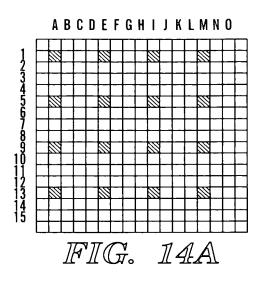
$$-OH + ClCH_2 \longrightarrow + HO- \longrightarrow -OCH_2CH(OH)CH_2O-$$

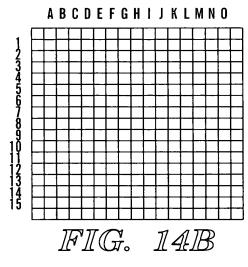
$$(NH-)$$

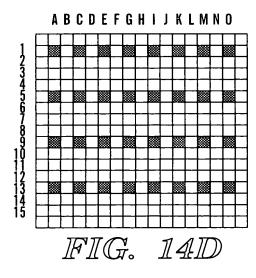
 $-OH \rightarrow -OCH_2(C=O)H + H_2N - + NaCNBH_3 \rightarrow -OCH_2CH_2NH -$

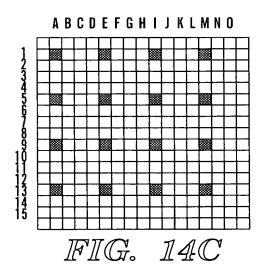












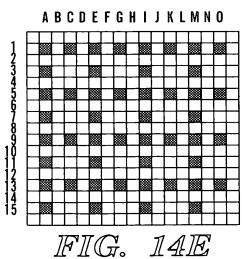


FIG. 15A

1st addition of unique 24mers.

FIG. 15B

2nd addition of unique 24mers.

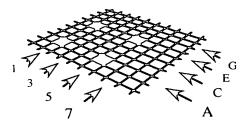
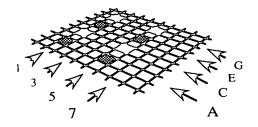
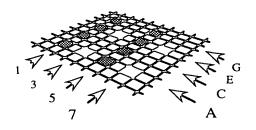
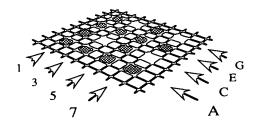


FIG. 15C 3rd addition of unique 24mers.



 $\mathbb{F}\mathbb{I}\mathbb{G}$, $\mathbb{I}5\mathbb{D}$ 4th addition of unique 24mers.





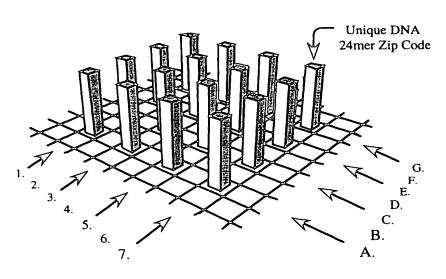
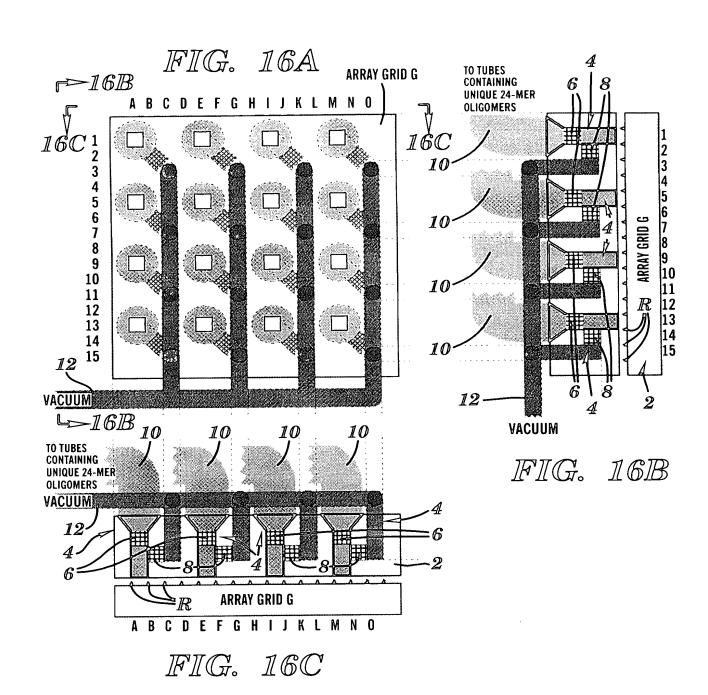


FIG. 15E





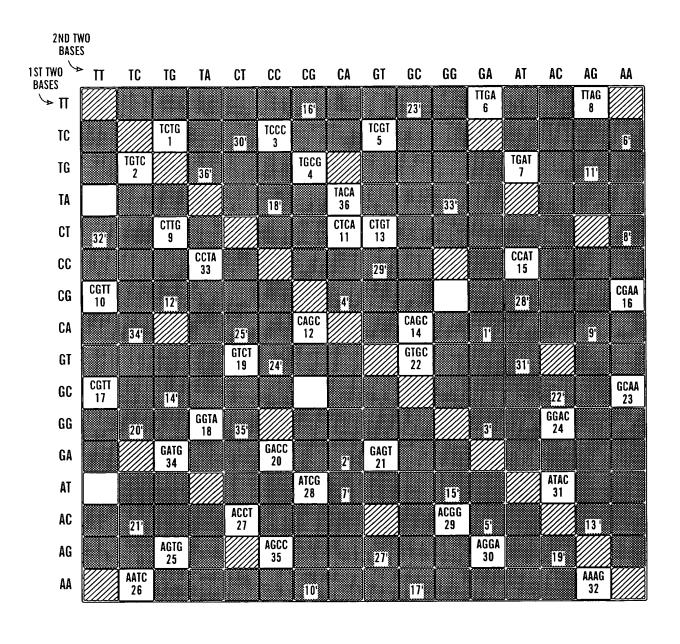
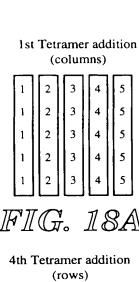


FIG. 17



2 2 6 6 6 5 5

(columns) FIG. 18A 4th Tetramer addition

FIG. 18D

18/34

2nd Tetramer addition (rows)

6	6	6	6	6	-
5	5	5	5	5	=
					_
4	4	4	4	4	_
3	3	3	3	3	_
2	2	2	2	2	

FIG. 18B

5th Tetramer addition

(columns)									
6		1		2		3		4	
6		1		2		3		4	l
6		1		2		3		4	
6		lı		2		3	۱.	4	l

FIG. 18E

3rd Tetramer addition (columns)

3	4	5	6	1
3	4	5	6	1
3	4	5	6	1
3	4	5	6	1
3	4	5	6	1

FIG. 18C

6th Tetramer addition (rows)

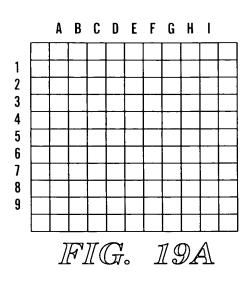
3	3	3	3	3
2	2	2	2	2
1	1	1	1	1
6	6	6	6	6
5	5	5	5	5

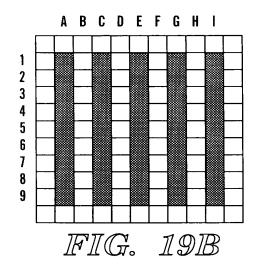
FIG. 18F

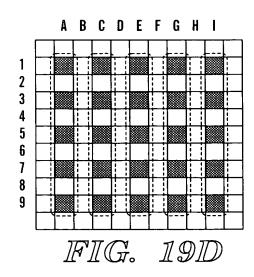
Addressable array with full length PNA 24mers

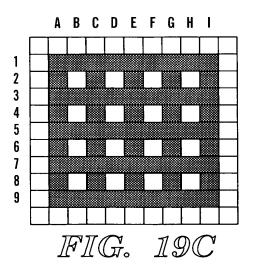
	-	_			
1-6-3-2-6-3	2-6-4-2-1-3	3-6-5-2-2-3	4-6-6-2-3-3	5-6-1-2-4-3	
1-5-3-1-6-2	2-5-4-1-1-2	3-5-5-1-2-2	4-5 - 6-1-3-2	5-5-1-1-4-2	
1-4-3-6-6-1	2-4-4-6-1-1	3-4-5-6-2-1	4-4-6-6-3-1	5-4-1-6-4-1	
1-3-3-5-6-6	2-3-4-5-1-6	3-3-5-5-2-6	4-3-6-5-3-6	5-3-1-5-4-6	
1-2-3-4-6-5	2-2-4-4-1-5	3-2-5-4-2-5	4-2-6-4-3-5	5-2-1-4-4-5	

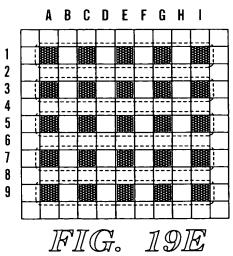
FIG. 18G

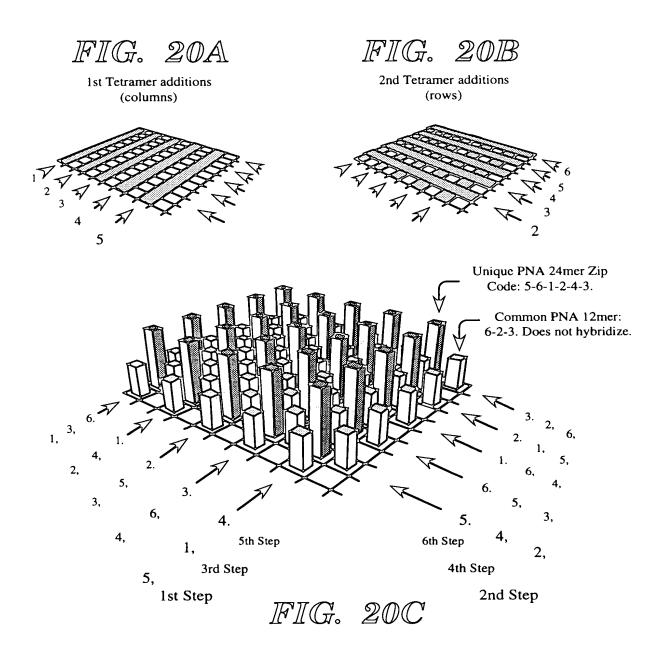


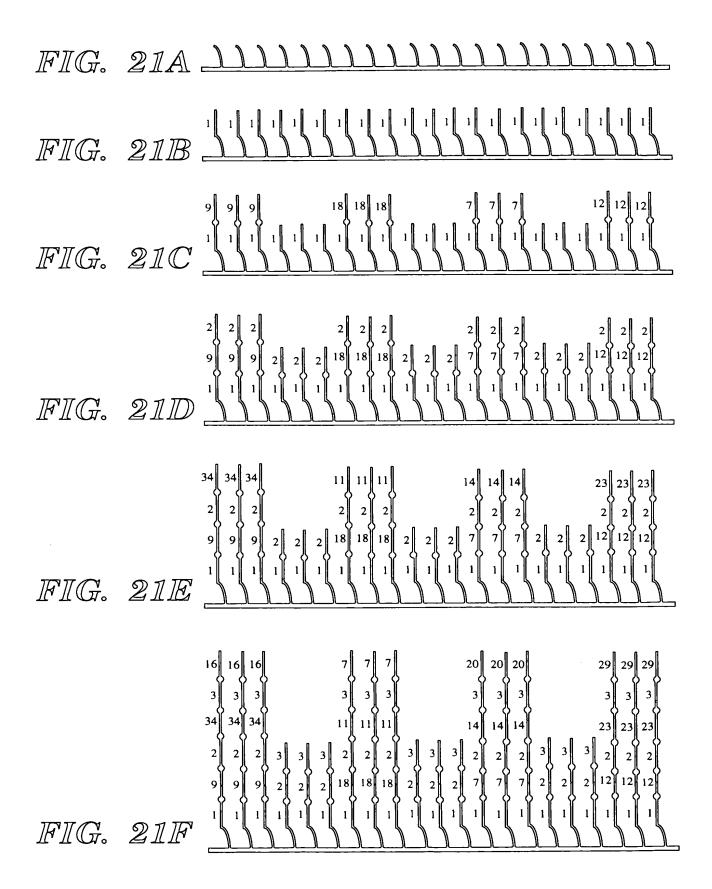


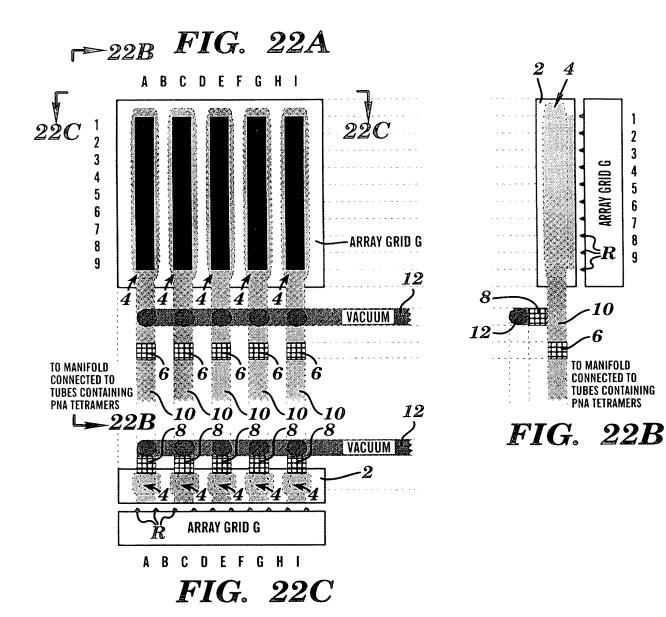


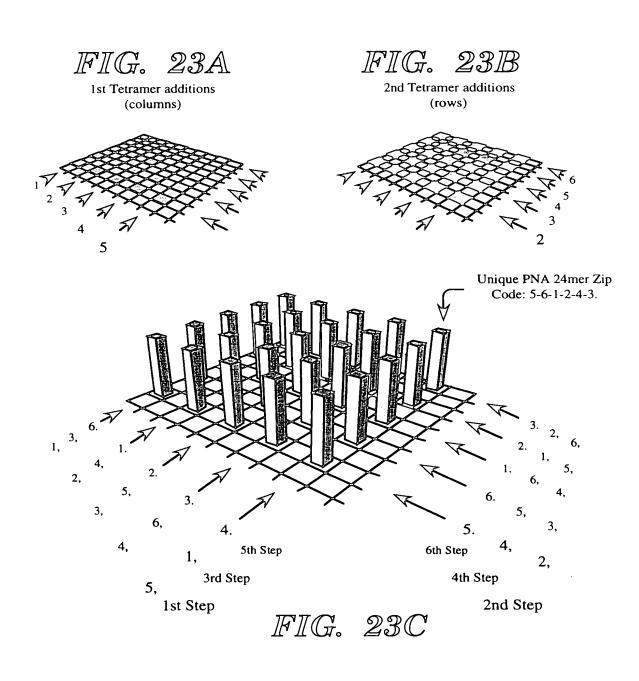






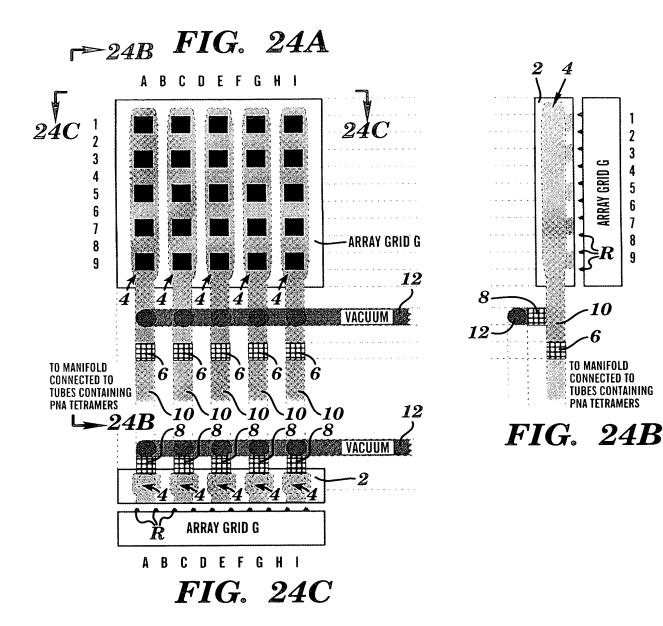


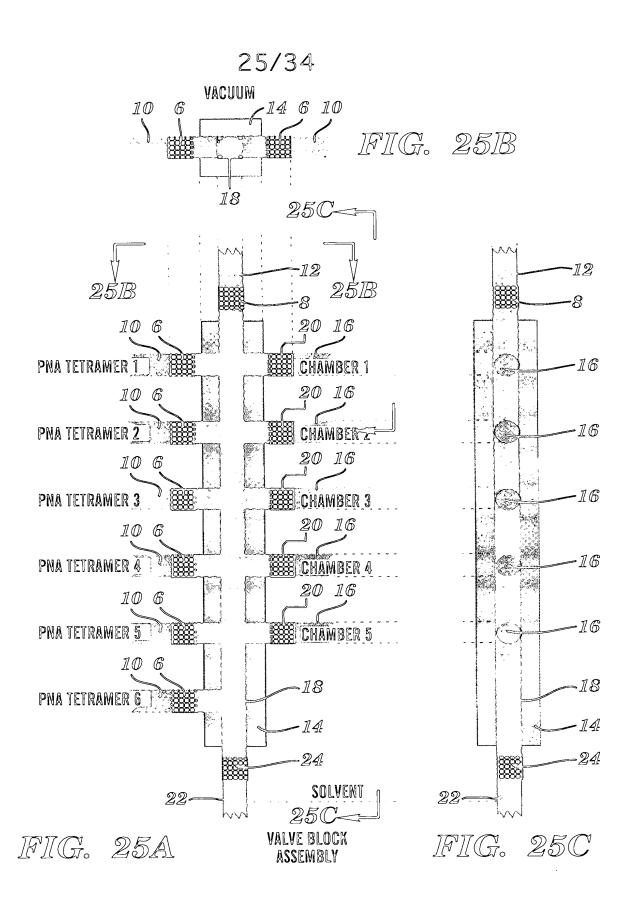




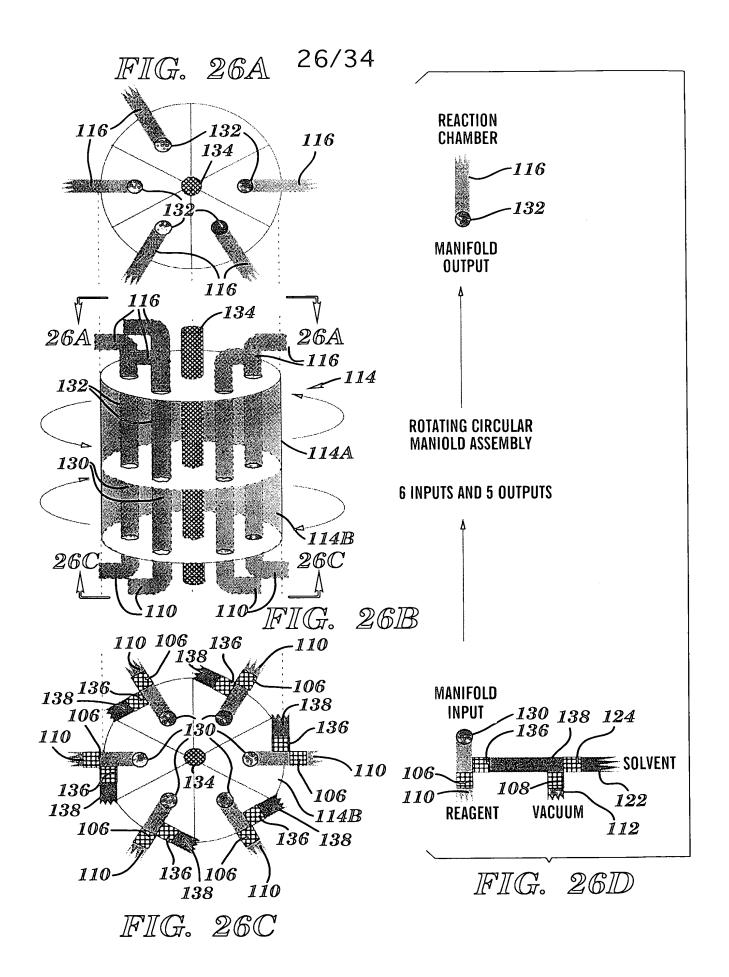
2

7





6 INPUTS AND 5 OUTPUTS



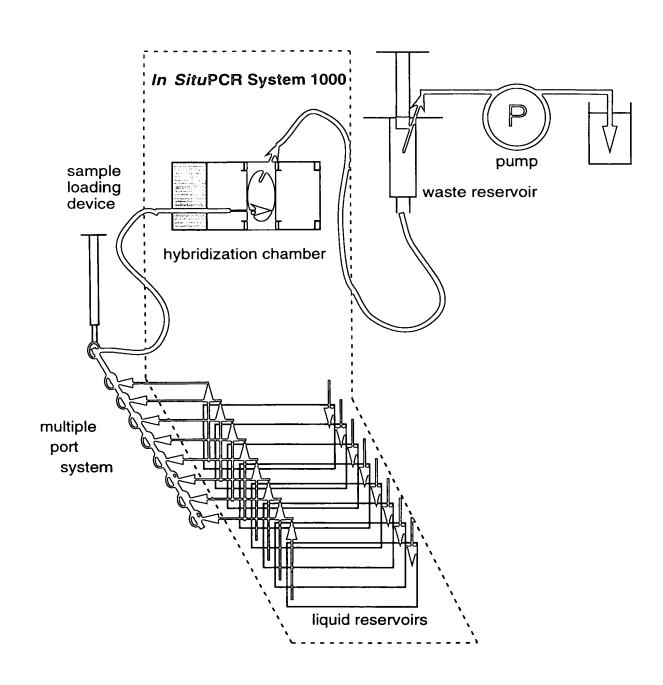


FIG. 27

-C00H; PR0BE 12

-COOH; PROBE 14

-NH2; PROBE 12

-NH2; PROBE 14

2% EGDMA

2% HDDMA

4% EGDMA

1 **(*)** 2 **(*)**

FIG. 31

FIG. 32

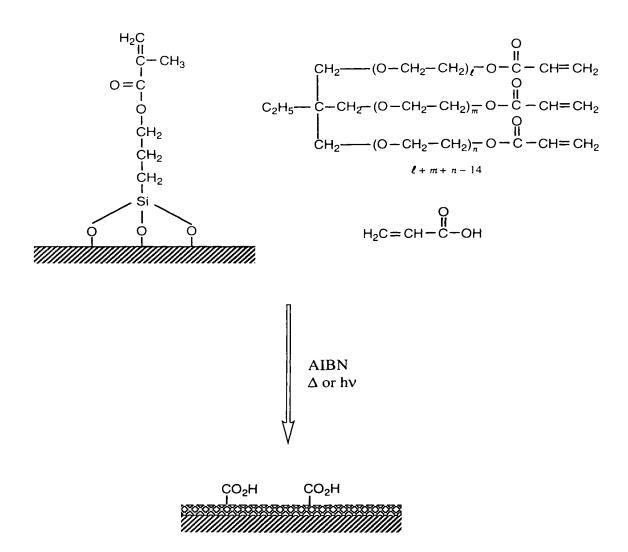


FIG. 33

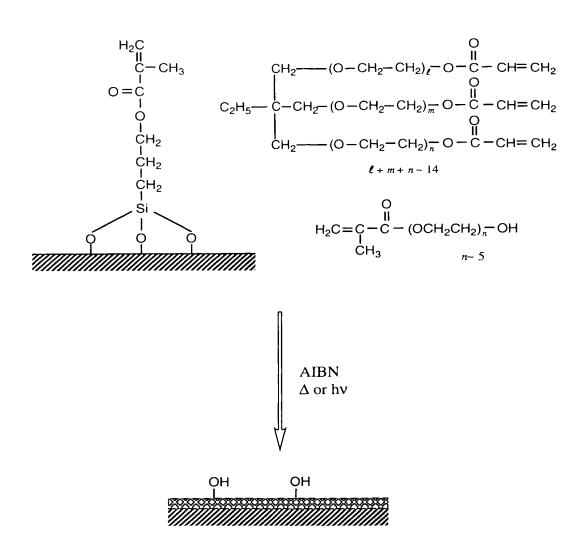


FIG. 34